CompCytogen 7(2): 103–110 (2013)
doi: 10.3897/CompCytogen.v7i2.4901
www.pensoft.net/journals/compcytogen

RESEARCH ARTICLE

COMPARATIVE Cytogenetics
A peer-reviewed open-access journal
International Journal of Plant & Animal Cytogenetics, Karyosystematics, and Molecular Systematics

Cytogenetics of the Porthole Shovelnose Catfish, *Hemisorubim platyrhynchos* (Valenciennes, 1840) (Siluriformes, Pimelodidae), a widespread species in South American rivers

Ana Cláudia Swarça[1], Sebastian Sanchez[2], Ana Lucia Dias[3], Alberto Sergio Fenocchio[4]

1 *Departamento de Histologia, CCB, Universidade Estadual de Londrina, CEP 86051-970, Caixa Postal 6001, Londrina, Paraná, Brazil* **2** *Instituto de Ictiología del Nordeste. Facultad de Ciencias Veterinarias. Universidad Nacional del Nordeste. Sargento Cabral 2139. 3400 Corrientes, Argentina* **3** *Departamento de Biologia Geral, CCB, Universidade Estadual de Londrina, CEP 86051-970, Caixa Postal 6001, Londrina, Paraná, Brazil* **4** *Universidad Nacional de Misiones, Departamento de Genética, Félix de Azara 1552. 3300 Posadas, Misiones, Argentina*

Corresponding author: *Ana Cláudia Swarça* (swarca@uel.br)

Academic editor: *A. Boron* | Received 21 February 2013 | Accepted 18 March 2013 | Published 25 April 2013

Citation: Swarça AC, Sanchez S, Dias AL, Fenocchio AS (2013) Cytogenetics of the Porthole Shovelnose Catfish, *Hemisorubim platyrhynchos* (Valenciennes, 1840) (Siluriformes, Pimelodidae), a widespread species in South American rivers. Comparative Cytogenetics 7(2): 103–110. doi: 10.3897/CompCytogen.v7i2.4901

Abstract

Hemisorubim platyrhynchos is a medium- to large-sized pimelodid catfish distributed along several river basins of the Neotropical Region, noteworthy for representing an important fishery source. In this work, *H. platyrhynchos* from three isolated populations were cytogenetically analyzed. The karyotype shows a diploid number of 2n=56 chromosomes comprising 22m, 16sm, 10st, 8a (FN=104). NORs detected by $AgNO_3$ were located in the terminal regions of the short arm of a st chromosome pair, as confirmed by CMA_3 and FISH using an 18S rDNA probe. C-banding revealed a small amount of heterochromatin in chromosomes, including the NORs, and one biarmed pair that showed conspicuous positive bands on both arms. This fact was also evidenced when using other banding techniques, such as RE (*Alu*I), and indicates that this pair constitutes a species-specific cytogenetic marker.

Keywords

Hemisorubim platyrhynchos, Pimelodidae, Cytogenetics

Copyright *Ana Cláudia Swarça et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License 3.0 (CC-BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Hemisorubim platyrhynchos (Valenciennes, 1840), popularly called "jurupoca" or porthole shovelnose catfish, is a pimelodid fish inhabiting the deeper and slow-moving sections of large South American rivers (Burgess 1989, Froese and Pauly 2012). This species is the sixth largest pimelodid of the Pantanal region/Brazil (Penha et al. 2004) and is considered an important species in the fisheries of the Paraná River basin (Agostinho et al. 1995). Its body shape and color pattern are adapted to the muddy bottom where it stays. It feeds on benthic organisms and fish (Froese and Pauly 2012). Breeding and sexing information have not been reported. The extensive exploitation of its populations due to overfishing could affect its genetic variability, which is still poorly known.

The family Pimelodidae represents one of the most specious catfish groups, however relationships among species of this group still remain as unanswered questions; however, it seems self-evident that they share certain characteristics (Nelson 2006, Ferraris 2007). Some authors has been divided this family in "groups" i.e. *Calophysus* Müller and Troschel in Müller 1843, *Pimelodus* LaCépède, 1803 and "Sorubiminae" (de Pinna 1998).

From a cytogenetic point of view some reports show that these groups could also share cytogenetic characteristics, supporting additionally the classification above mentioned (Swarça et al. 2007, Sanchez et al. 2010, Carvalho et al. 2011).

Hemisorubim platyrhynchos is a monotypic species that belongs to the family Pimelodidae, however, it is considered one of the "sorubimine catfishes", an informal group of catfish that comprises other genera such as *Sorubim* Cuvier, 1829, *Pseudoplatystoma* Bleeker, 1862, and *Brachyplatystoma* Bleeker, 1862 (Lundberg and Akama 2005).

Until now only one population of *H. platyrhynchos* of the Parana River (Brazil) has been cytogenetically studied and has had its diploid number, AgNORs location and C-banding reported (Martins-Santos et al. 1996).

The objective of the present study was to describe the karyotypic structure of specimens from three populations of *H. platyrhynchos* aiming to characterize and compare the obtained results with the available cytogenetic data on this and other related species.

Material and methods

Fifteen specimens of *Hemisorubim platyrhynchos* consisting of 8 males (m) and 5 females (f), caught in the Parana River (Corrientes State, Argentina) and 2 specimens of undetermined sex from the Miranda River (Mato Grosso do Sul State, Brazil) were cytogenetically analyzed. The sampling sites in the Paraná River were: Ituzaingó (2 m), Itá Ibaté (2 f - 3 m), Yahapé (1 m), Puerto Abra (1 f), and Corrientes (2 f / 2 m) (Corrientes Province). Mitotic chromosome preparations were obtained according to the technique described by Foresti et al. (1993) for some specimens of Paraná River/Argentina and from blood culture for specimens of the Miranda River/Brazil (Fenocchio and Bertollo 1988). The specimens were deposited in the collection of the Laboratory of

the Instituto de Ictiología del Nordeste, Universidad Nacional del Nordeste/Argentina. Nucleolus organizer regions (Ag-NORs) were revealed by the silver-staining method (Howell and Black 1980) and C-banding was performed according to Sumner (1972). Restriction endonucleases were used according to Sánchez et al. (1990) with some modifications, i.e., *Alu.* I was used a concentration of 0.3 U/μL and the chromosome preparations were treated at 37°C for 4h. Chromosome staining with Chromomycin A_3 (CMA_3), a C-G specific fluorochrome, was applied according to the description provided by Verma and Babu (1995). Fluorescent *in situ* hybridization (FISH) was carried out by means of biotinylated 18S rDNA probes (segment with 1700 pb) obtained from the nuclear DNA of the fish *Oreochromis niloticus* (Linnaeus, 1758) labeled with biotin-14-dATP by nick translation (Gibco cat Nº 18247-015), according to the manufacturer's instructions. The hybridization technique, post-hybridization washes and visualization were carried out as reported by Swarça et al. (2001). The chromosomes were classified according to their arm ratios as metacentrics (m), submetacentrics (sm), subtelocentrics (st), and acrocentrics (a) (Levan et al. 1964, Guerra 1986) with modifications. The m, sm, st were considered as biarmed chromosomes and acrocentrics were considered as uniarmed chromosomes.

Results

All three populations of *H. platyrhynchos* presented the same results. The diploid number obtained was 2n=56 and the karyotype was composed of 22m+16sm+10st+8a (NF=104) without chromosomal differences between sexes (Fig. 1).

The AgNORs were located in the terminal position on the short arm of a subtelocentric (st) pair (Figs 2a, b). The bright signals correspond to the zones evidenced by argentic impregnation after FISH with the 18S rDNA probe and staining with CMA_3 (Figs 2c, d). This chromosome pair is clearly identified due to its size, shape and exclusive secondary constriction. C-banding revealed positive bands in the pericentromeric

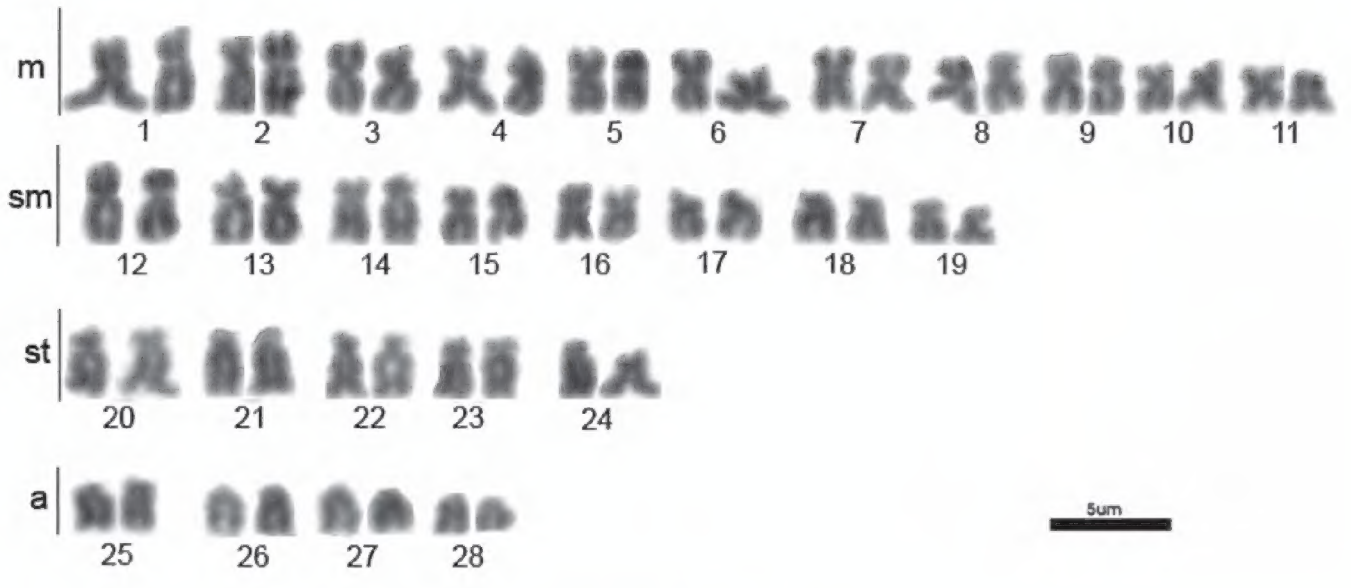

Figure 1. Karyotype of *Hemisorubim platyrhynchos*. Bar = 5mm.

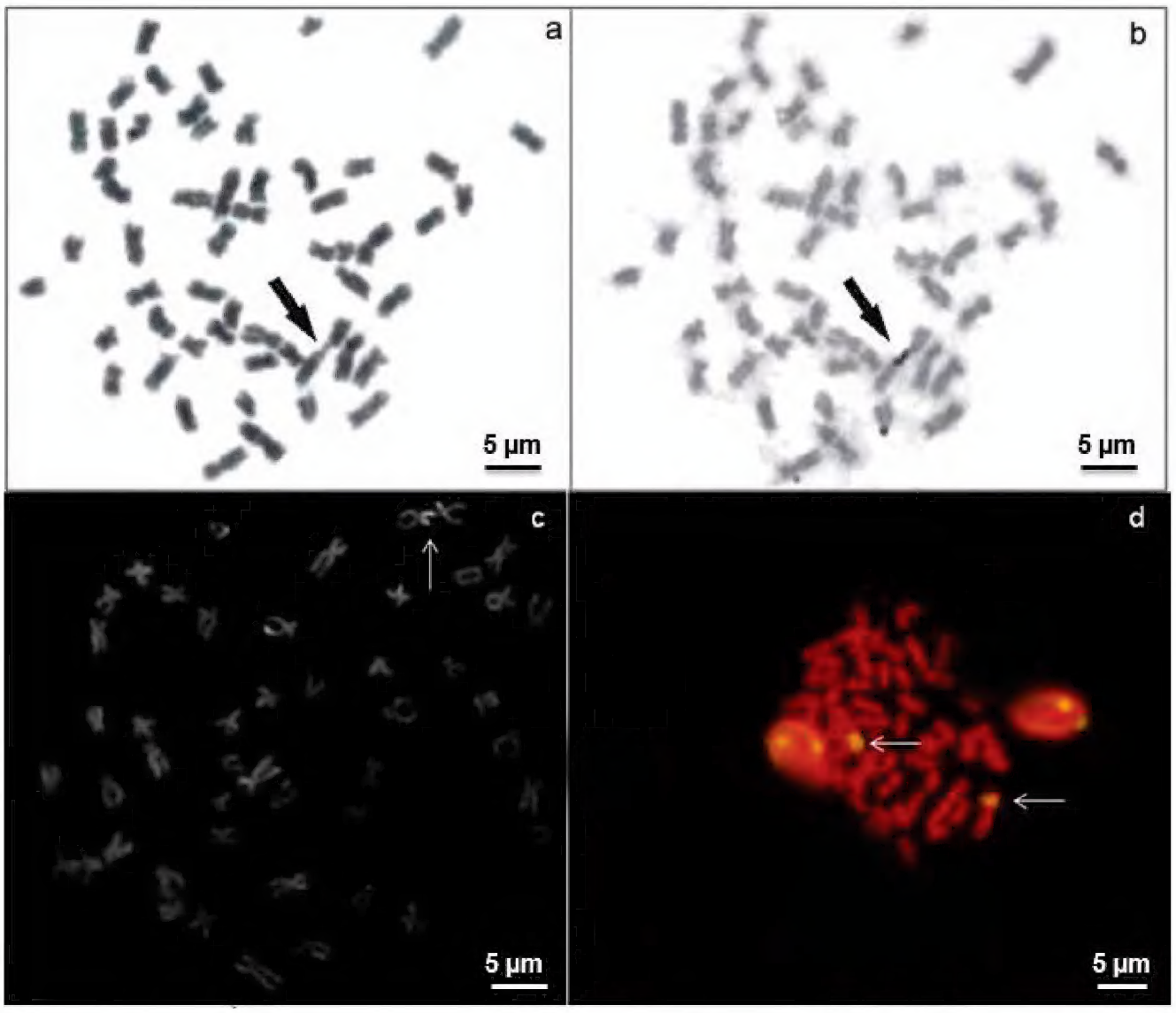

Figure 2. Metaphases of *Hemisorubim platyrhynchos* showing sequential Giemsa-$AgNO_3$ staining (**a, b**) CMA_3 banding (**c**) FISH with 18S rDNA probe (**d**). Arrows indicate the NOR- bearing chromosomes.

regions of some chromosome pairs and on the short arms of a st chromosome pair, coincident with positive C-bands and allowed the identification of a large biarmed marker pair with positive bands on almost the entire short and long arms (Fig. 3b). The *Alu*I restriction enzyme shows bands that resemble C-banding, principally on the biarmed chromosome, producing a reverse pattern (Fig. 3c). The mentioned chromosome could be considered a species-specific cytogenetic marker, since it has not been reported in other species of this group of fish.

Discussion

The karyotype of *H. platyrhynchos* was composed of 22m+16sm+10st+8a (NF=104), however, despite having the same diploid chromosome number 2n=56, *H. platyrhynchos* from the Paraná River/Brazil reported by Martins-Santos et al. (1996) presented 22m + 18sm + 6st + 10a (NF=102, recalculated in the present paper). These variations could be ascribed to chromosome rearrangements, although when alternatively using parsimony criteria, differences could be attributed to the condensation of the chromosome arms and/or to technical artifacts. In the family Pimelodidae, 23 of the 27

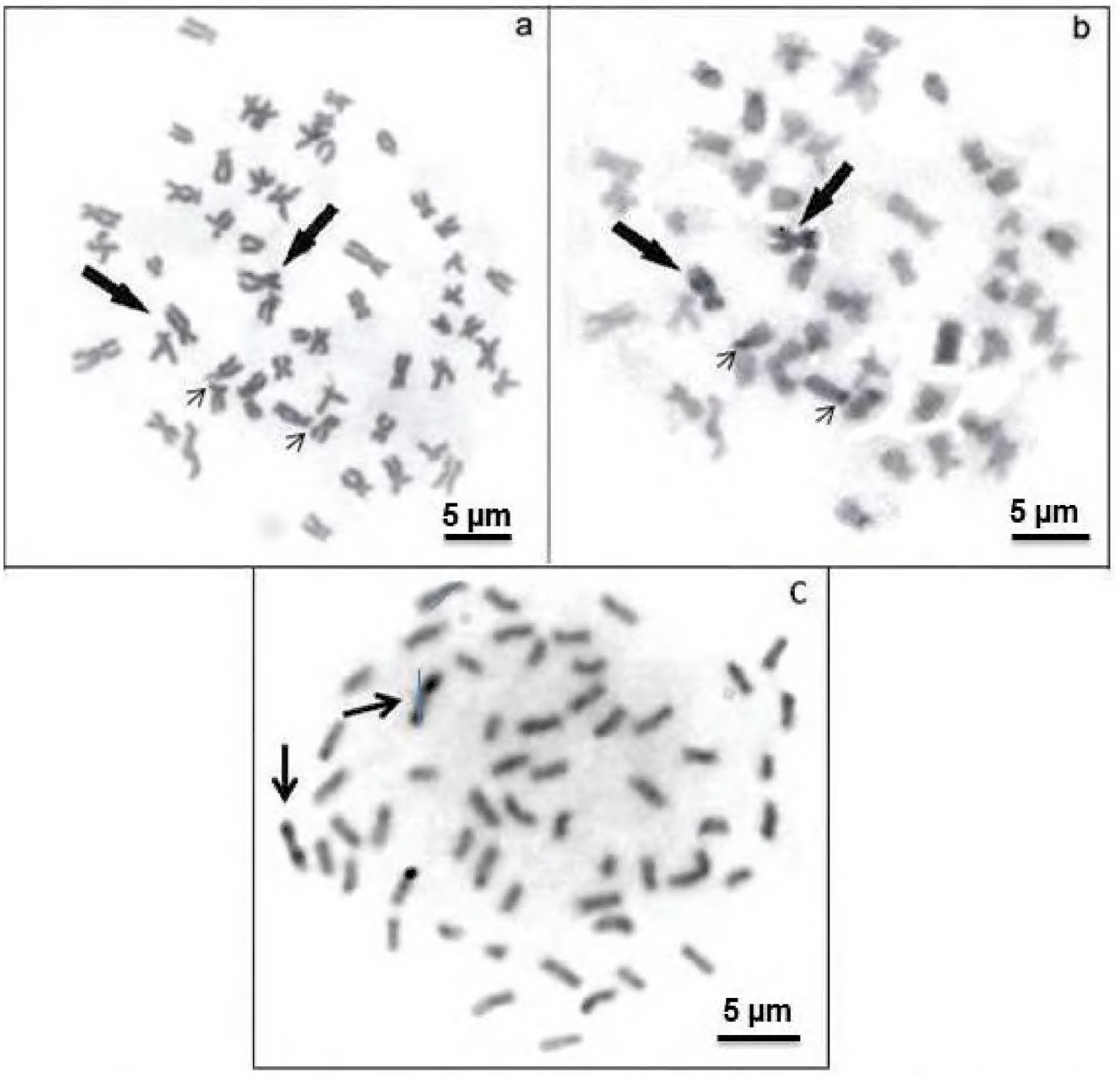

Figure 3. Metaphases of *Hemisorubim platyrhynchos* showing sequential Giemsa-C banding (**a, b**) and after *Alu*I treatment (**c**). The arrows indicate the biarmed chromosome pair (marker) with positive bands on the short and long arms, the thin arrows show the NOR-bearing chromosomes.

karyotyped species have a diploid number of 2n= 56 chromosomes, except for *Calophysus macropterus* Lichtenstein, 1819, *Luciopimelodus pati* (Valenciennes, 1840), and *Pinirampus pirinampu* (Spix & Agassiz, 1829) with 2n=50 and *Megalonema platanum* (Günther, 1880) with 2n=54, which seem to share other characteristics (Swarça et al. 2007).

One point worth emphasizing is the homogeneity of the karyotypes of species belonging to the "Sorubiminae group" with a clear prevalence of biarmed chromosomes, showing a high fundamental number. A cytogenetic feature shared by all species of this group is the AgNORs localized in the terminal position on the short arm of one pair of st/a chromosomes that also could be evidenced by C-banding, as observed in the present study and in other studied species, such as *Sorubim lima* (Bloch & Schneider, 1801) (Fenocchio and Bertollo 1992, Martins-Santos et al. 1996), *P. corruscans* (Spix & Agassiz, 1829) and *P. tigrinus* (Valenciennes, 1840) (Fenocchio and Bertollo 1992, Martins-Santos et al. 1996, Swarça et al. 2005a), *Zungaro zungaro* (Humboldt, 1821)

(Martins-Santos et al. 1996, Swarça et al. 2001), *Steindachneridion scriptum* (Miranda-Ribeiro, 1918) (Swarça et al. 2005b) and *Steindachneridion sp* (=*melanodermatum*) (Garavello, 2005) (Swarça et al. 2006).

CMA_3 staining and FISH with 18S rDNA exhibited fluorescent signals that correspond to the AgNOR sites (Fig. 2c, d). This correspondence between AgNORs, C-banding, FISH and CMA_3 staining has already been observed in almost all species of the Pimelodidae family (Swarça et al. 2001, 2008).

The relatively low amount of heterochromatin in chromosomes of *H. platyrhynchos* and in other species of the Pimelodidae catfishes suggests that this may be a characteristic of this family. On the other hand, C-banding allowed the identification of a large biarmed pair with positive bands on almost the entire short arm and on the long arm. The *Alu*I restriction enzyme on fish chromosomes produces a C-banding-like pattern (Maistro et al. 2000) and this was also observed in *H. platyrhynchos* chromosomes (Fig. 3c). This chromosome pair could be considered a species-specific cytogenetic marker, as it has not been reported in other Pimelodidae (Fig. 3b).

According to cytogenetic traits, this family could be divided into two: the "*Pimelodus* group" and the "Sorubiminae group" (=Sorubinae), and the cytogenetic data confirm that the analyzed species belongs to the second group, because it has 2n=56 chromosomes, a high NF and the NORs localized on one single chromosome pair in the terminal position of the short arms, as it occurs with the other species of this group (Swarça et al. 2007). Thus, despite its wide geographic distribution, it is evident that *H. platyrhynchos* shows a marked conservatism in its basic karyotype macrostructure, differing from many species of the Pimelodidae family, i.e., the "*Pimelodus* group", which presents a wide karyotypic variability, even within the same hydrographic basin and within the same river.

References

Agostinho AA, Vazzoler AEAM, Thomaz S (1995) The high Paraná river basin: limnological and ichthyological aspects. In: Tundisi JG, Bicudo EM, Matsumura-Tundisi T (Eds) Limnology in Brazil. Brazilian Academy of Sciences, Brazilian Limnological Society, Rio de Janeiro, 59–103.

Burgess WE (1989) An atlas of freshwater and marine catfishes: a preliminary survey of the Siluriformes. T.F.H. Publications, New Jersey, 784 pp.

Carvalho R, Sànchez S, Swarça AC, Fenocchio AS, Martins-Santo IC, Dias AL (2011) Chromosomal analyses in *Megalonema platanum* (Siluriformes: Pimelodidae), an endangered species from South American rivers. Neotropical Ichthyology 9 (1): 177–182. doi: 10.1590/S1679-62252011005000008

Derenzini M, Hernandéz-Verdún D, Farabegoli F, Pession A, Novello F (1987) Structure of ribosomal genes of mammalian cells *in situ*. Chromosoma 95:63–70. doi: 10.1007/BF00293843

de Pinna MCC (1998) Phylogenetic relationships of Neotropical Siluriformes (Teleostei: Ostariophysi); historical overview and synthesis of hypothesis. In: Malabarba LR, Reis RE, Vari RP, Lucena ZM, Lucena CAS (Eds) Phylogeny and Classification of Neotropical Fishes. Edipucrs, Porto Alegre, Brazil, 279– 330.

Fenocchio AS, Bertollo LAC (1988) A simple method for fresh-water fish lymphocyte culture. Brazilian Journal of Genetics 11(4): 847–852.

Fenocchio AS, Bertollo LAC (1992) Karyotype similarities among Pimelodidae (Pisces, Siluriformes) from the Brazilian Amazon region. Cytobios 69: 41-46.

Ferraris CJ (2007) Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of Siluriform primary types. Zootaxa 1418: 1–628.

Foresti F, Oliveira C, Almeida-Toledo LF (1993) A method for chromosome preparations from large specimens of fishes using in vitro short treatment with colchicine. Experientia 49: 810–813. doi: 10.1007/BF01923555

Froese R, Pauly D (Eds) (2012) FishBase. World Wide Web Electronic Publication. Available at http://www.fishbase.org, version (04/2012). [accessed on February 05, 2013]

Guerra M (1986) Reviewing the chromosome nomenclature of Levan et al. Brazilian Journal of Genetics 4: 741–746.

Howell WM, Black DA (1980) Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a one step method. Experientia 36:1014–1015. doi: 10.1007/BF01953855

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52: 2101–2220.

Lundberg JG, Akama A (2005) *Brachyplatystoma capapretum*: a New Species of Goliath Catfish from the Amazon Basin, with a Reclassification of Allied Catfishes (Siluriformes: Pimelodidae). Copeia 2005 (3): 492–516. doi: 10.1643/CI-04-036R1

Maistro EL, Oliveira C, Foresti F (2000) Cytogenetic analysis of A- and B-chromosomes of *Prochilodus lineatus* (Teleostei, Prochilodontidae) using different restriction enzyme banding and staining methods. Genetica 108: 119–125. doi: 10.1023/A:1004063031965

Martins-Santos IC, Julio Jr HF, Burin I (1996) Karyotypic studies of four species of the Sorubiminae subfamily (Pisces, Siluriformes). Caryologia 49: 73–80.

Nelson JS (1994) Fishes of the World, 3rd ed. Wiley, New York, 600pp.

Penha JM, Mateus LA, Barbieri G (2004) Age and growth of the porthole shovelnose catfish (*Hemisorubim platyrhynchos*) in the Pantanal. Brazilian Journal of Biology 64(4): 833–40. doi: 10.1590/S1519-69842004000500013

Sànchez L, Martinez P, Vinas A, Bouza C (1990) Analysis of the structure and variability of nucleolar organizer regions of *Salmo trutta* by C-, Ag-, and restriction endonuclease banding. Cytogenetic and Cell Genetic 54: 6–9. doi: 10.1159/000132944

Sànchez S, Swarça AC, Fenocchio AS (2010) Cytogenetic Studies and Evolutive Considerations in Species of the *Calophysus* Group (Siluriformes, Pimelodinae). Cytologia 75(3): 223–227. doi: 10.1508/cytologia.75.223

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research 75: 304–306. doi: 10.1016/0014-4827(72)90558-7

Swarça AC, Cestari MM, Giuliano-Caetano L, Dias AL (2001) Cytogenetic characterization of the large south American Siluriform fish species *Zungaro zungaro* (Pisces, Pimelodidae). Chromosome Science 5: 51–55.

Swarça AC, Fenocchio AS, Cestari MM, Dias AL (2005a) Chromosomal divergence among populations of *Pseudoplatystoma corruscans* (Pisces, Pimelodidae): I. Evidences obtained from conventional Giemsa staining, C- and G- banding. Ichthyological Exploration Freshwaters 16(4): 325–330.

Swarça AC, Fenocchio AS, Cestari MM, Dias AL (2005b) First chromosome data on *Steindachneridion scripta* (Pisces, Siluriformes, Pimelodidae) from Brazilian Rivers: Giemsa, CBG, G- and RE banding. Genetic and Molecular Research 4(4): 734–741.

Swarça AC, Fenocchio AS, Cestari MM, Dias AL (2006) First report of a sex chromosome system in a Sorubiminae fish, *Steindachneridion* sp. (Pimelodidae), with an uncommon large Y chromosome. Mitotic and Meiotic analysis. Cytogenetic and Genome Research 112(3-4): 325–328. doi: 10.1159/000089888

Swarça AC, Fenocchio AS, Dias AL (2007) An update Cytogenetic Review for Species of the Families Pseudopimelodidae, Pimelodidae and Heptapteridae (Pisces, Siluriformes). Suggestion of a Cytotaxonomical Classification. Caryologia 60(4): 338–348.

Swarça AC, Fenocchio AS, Cestari MM, Dias AL (2008) Analyses of the Structure of NORs in Two Species of South American Sorubiminae Fishes (Siluriformes) by Means of Several Cytogenetic Techniques. Folia Biologica (Kraków) 56 (1-2): 31–35. doi: 10.3409/fb56_1-2.31-35

Verma RS, Babu A (1995) Human Chromosomes: Principles and Techniques. 2nd ed. United States of America. International Edition, 419 pp.